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Patentanmeldung Nr. | Patent application No. | Demande de brevet n°

02251605.8

PRIORITY DOCUMENT

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Der Präsident des Europäischen Patentamts; Im Auftrag

For the President of the European Patent Office

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Blatt 2 der Bescheinigung
Sheet 2 of the certificate
Page 2 de l'attestation

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COMPOSITIONS OF POLYMERS

The present invention relates to aqueous compositions comprising polymers and biologically active compounds, especially polymeric drug delivery systems.

DNA delivery has been a flagship in non-viral gene delivery because the promise of therapeutic DNA delivery as a potential cure for many genetic diseases has stimulated much interest over the past decade. With unacceptable immune responses and other adverse events recently reported for viral delivery, non-viral gene delivery becomes even more attractive. However, the main limitation with non-viral delivery is the inefficient transfection, caused mainly by the poor transport of DNA across cell membranes. Various cationic polyelectrolytes have shown promising effects in facilitating gene delivery as these polymers readily conjugate with DNA to neutralize the net negative charges from DNA molecules. However, recent research has indicated that successful polymeric candidates much satisfy a set of requirements. First, although neutralization helps to mediate negative charges in DNA and improve the transport, the polymer must not destabilize the helical structures so that its bioactivity is lost. In addition, it must not impose cytotoxicity to cells either. Cationic polymers such as poly-L-lysine (PLL), polyethylenimine (PEI) and polyamidoamine dendrimer all readily from molecular conjugates with DNA. Unfortunately, high toxicity has been reported from these polymers, which is associated with the dissociation of protons on the primary and secondary amine side chains (the so called proton sponge effect). This event occurs in the endosomal compartment of cell, which activates the complement system leading to cell death. Second, Langer *et al* have demonstrated that for effective gene delivery, the size of conjugated particles must not be over 150 nm for them to be engulfed by cells Pack, Putnam, Langer, *Biotech. Bioeng.* 2000, 67, 217. Third, the conjugated particles must be readily dispersable in aqueous solution. Unstable aggregates are difficult to administer and are rapidly cleared from systemic administration. These conditions together with the level of the high

cost in the synthesis of some of the cationic polymers mean that few existing polymers can meet this set of requirements.

Copolymers of tertiary amine alkyl methacrylates with polyethylene glycol have been investigated for their potential to serve as vectors for gene therapy, by Rungfardthong, U. *et al* J. Contr. Rel. (2001), 73(2-3), 359-380. Polymers investigated included low polydispersity block copolymers as well as comb polymers formed by statistical copolymerisation of the tertiary amine alkyl methacrylate and a mono-methacrylated oligo(ethyleneglycol) monomer. The incorporation of the PEG moieties enabled colloidally stable complexes of polymer and DNA to be generated. *In vitro* transfection experiments showed some transfection took place, albeit that lower levels than a control poly-L-lysine system.

In WO-A-99/06055 block copolymers comprising a non-ionic block and a cationic block are used to deliver nucleic acids. The non-ionic block may comprise polyacrylamide. The cationic block may comprise polyethylene imine, or a polyimine polymer formed from dibromobutane and N-(3-aminopropyl)-1,3-propane diamine, or a lysine polymer or copolymer (with alanine). Polynucleotide complexed with the copolymers were protected from nuclease attack, and were successful in *in vivo* and *in vitro* transfection experiments.

Cationic drugs have been delivered using systems based on anionic polymers, for instance block copolymers comprising non-ionic blocks and anionic blocks. Govender, T. *et al* in J. Contr. Rel. (2001), 75(3), 249-258 describe non-covalent interactions between a poly(aspartic acid)-poly(ethylene glycol) block copolymer with diminazene aceturate, a low molecular weight cationic drug.

Bronich, T. K. *et al* in Langmuir (2000), 16(2), 481-489, describe block copolymers of polyethylene oxide and poly(sodium methacrylate) with cationic surfactants such as cetylpyridinium bromide. The complexes formed stable dispersions with particle sizes in the range 100 to 200 nm. The authors describe the effect of changing the block length of the PEO block,

and of the sodium methacrylate block on the properties of the dispersion. Similar disclosures are in WO-A-98/56348 and WO-A-98/56334.

In our earlier patent publications WO-A-98/22516 and WO-A-98/22517 we describe polymers, primarily used for coating substrates, having pendant cationic and zwitterionic groups, used in conjunction with anionic biologically active materials such as anionic mucopolysaccharides, especially heparin. Although the polymer is generally coated onto a substrate, and the coated substrate subsequently contacted with the anionic active, it is also suggested that the polymer and active may be premixed in a compatible solvent, to form a coating solution.

In our earlier publications WO-A-00/28920 and WO-A-00/29481, we describe polyion complexes formed of oppositely charged polyelectrolytes, at least one of which has pendant zwitterionic groups. The polyion complexes may be used as drug delivery depots, although there are no examples of selection of specific polymers for use with specific actives.

In Langmuir (2000) 16, 5980-5986 Styrkas D.A. *et al.* describe adsorption at a solid-liquid interface of low polydispersity block copolymers formed of a tertiary amino alkylmethacrylate block and a sulphobetaine-group containing block. The adsorption showed pH-dependent effects which the authors compared to the pH dependent effects of micelle formation of these block copolymers described in earlier work by Bütün, V. *et al.* in J. Mater. Chem. (1997), 7, 1693.

A new liquid aqueous composition according to the invention comprises a suspension of a polymer having an overall ionic charge and a biologically active compound having a charge opposite that of the polymer and is characterised in that the polymer has pendant zwitterionic groups.

The invention is of most value where the biologically active compound is anionic, preferably polyanionic, in nature. The invention is of most value where the active compound is a nucleic acid, for instance an oligonucleotide, having 5 to 50 base residues usually of DNA. For instance the oligonucleotide may be an active anti-sense molecule. The nucleic acid may

alternatively be a single strand RNA molecule or a single or double strand DNA molecule. Double stranded DNA may, for instance, comprise genes encoding useful products, especially a plasmid, including control sequences enabling it to be transcribed and translated when transfected into a cell. The invention is thus usefully a gene delivery system. Other anionic actives may be saccharide-containing compounds, proteins or peptides and amphiphilic anionic compounds such as retinoic acid and derivatives.

The invention may also be useful where the biologically active compound is a cationic drug, especially a polycationic drug or an amphiphilic cationic drug. Examples are Cetyl and other long chain alkyl-pyridinium compounds, and anaesthetics, such as procaine-HCl, rhodamine probes, and low molecular weight drugs such as mexilitine, amiloride HCl, diminazene aceturate and amikicin sulphate.

The composition of the invention generally comprises polymer and biologically active compound associated with one another in the form of particles having an average diameter of less than 200 nm, preferably less than 150 nm. Particles of size less than the indicated maximum, are capable of being taken up by cells, so that the biologically active compounds may be delivered intracellularly. Such particles may also be stabilized against settlement in the aqueous composition. The composition thus retains useful rheology, enabling it to be handled by usual liquid handling techniques, without having to be thickened or gelled to stabilise the particles against settlement.

The particle size may depend upon the molecular size of the biologically active compound and/or of the copolymer. It will also depend upon other features of the copolymer, for instance the nature of the monomers from which the polymer is formed. Preferably the copolymer has a molecular weight (weight average) less than 500,000, preferably less than 100,000, for instance 50,000 Da.

The polymer of the invention may be a random (statistical) copolymer, for instance formed from a mixture of monomers, comprising zwitterionic

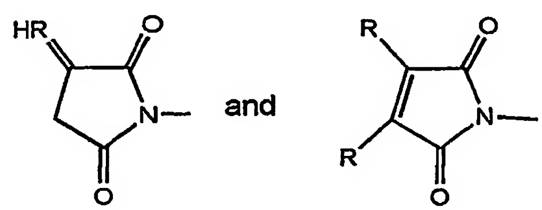
monomers and ionic or ionisable monomers. Statistical copolymers are generally amphiphilic in nature, that is comprise further hydrophobic moieties, particularly hydrophobic pendant groups. Such moieties are generally provided by the use of monomers having pendant hydrophobic groups. The polymer may, for instance, be a terpolymer as described in our earlier publication WO-A-98/22516. Preferably it is synthesised, however by a living radical polymerisation process, to provide low polydispersity polymer products. Suitable processes are atom or group transfer polymerisation processes described in more detail below.

Preferably, however, the polymer is a block copolymer comprising a zwitterionic block comprising the said pendant zwitterionic groups, and an ionic block, comprising the said ionic groups.

Generally the zwitterionic block is formed from ethylenically unsaturated monomers including a zwitterionic monomer having the general formula

$$YBX \qquad \text{I}$$

in which Y is an ethylenically unsaturated group selected from $H_2C{=}CR{-}CO{-}A{-}$, $H_2C{=}CR{-}C_6H_4{-}A^1{-}$, $H_2C{=}CR{-}CH_2A^2$, $R^2O{-}CO{-}CR{=}CR{-}CO{-}O$, $RCH{=}CH{-}CO{-}O{-}$, $RCH{=}C(COOR^2)CH_2{-}CO{-}O$,



A is -O- or NR^1;

A^1 is selected from a bond, $(CH_2)_lA^2$ and $(CH_2)_l\,SO_3$- in which l is 1 to 12;

A^2 is selected from a bond, -O-, O-CO-, CO-O, $CO{-}NR^1{-}$, $-NR^1{-}CO$, $O{-}CO{-}NR^1{-}$, $NR^1{-}CO{-}O{-}$;

R is hydrogen or C_{1-4} alkyl;

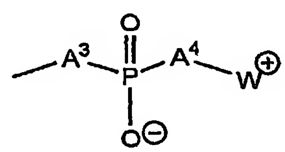
R^1 is hydrogen, C_{1-4} alkyl or BX.

R^2 is hydrogen or C_{1-4} alkyl;

B is a bond, or a straight branched alkanediyl, alkylene oxaalkylene, or alkylene (oligooxalkylene) group, optionally containing one or more fluorine substituents;

X is a zwitterionic group.

Preferably X is an ammonium, phosphonium, or sulphonium phosphate or phosphonate ester zwitterionic group, more preferably a group of the general formula II



II

in which the moieties A^3 and A^4, which are the same or different, are -O-, -S-, -NH- or a valence bond, preferably -O-, and W^+ is a group comprising an ammonium, phosphonium or sulphonium cationic group and a group linking the anionic and cationic moieties which is preferably a C_{1-12}-alkanediyl group,

preferably in which W^+ is a group of formula $-W^1-N^+R^3_3$, $-W^1-P^+R^4_3$, $-W^1-S^+R^4_2$ or $-W^1-Het^+$ in which:

W^1 is alkanediyl of 1 or more, preferably 2-6 carbon atoms optionally containing one or more ethylenically unsaturated double or triple bonds, disubstituted-aryl (arylene), alkylene arylene, arylene alkylene, or alkylene aryl alkylene, cycloalkanediyl, alkylene cycloalkyl, cycloalkyl alkylene or alkylene cycloalkyl alkylene, which group W^1 optionally contains one or more fluorine substituents and/or one or more functional groups; and

either the groups R^3 are the same or different and each is hydrogen or alkyl of 1 to 4 carbon atoms, preferably methyl, or aryl, such as phenyl, or two of the groups R^3 together with the nitrogen atom to which they are attached form an aliphatic heterocyclic ring containing from 5 to 7 atoms, or the three groups R^3 together with the nitrogen atom to which they are attached as heteroaromatic ring having 5 to 7 atoms, either of which rings may be fused with another saturated or unsaturated ring to form a fused ring

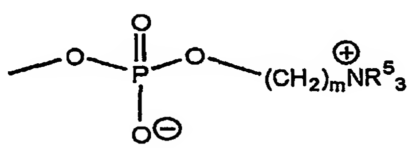
structure containing from 5 to 7 atoms in each ring, and optionally one or more of the groups R^3 is substituted by a hydrophilic functional group, and

the groups R^4 are the same or different and each is R^3 or a group OR^3, where R^3 is as defined above; or

Het is an aromatic nitrogen-, phosphorus- or sulphur-, preferably nitrogen-, containing ring, for example pyridine.

Monomers in which X is of the general formula in which W^+ is $W^1N^{\oplus}R^3_3$ may be made as described in our earlier specification WO-A-9301221. Phosphonium and sulphonium analogues are described in WO-A-9520407 and WO-A-9416749.

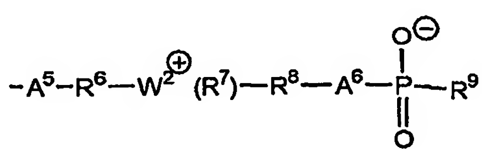
Generally a group of the formula II has the preferred general formula III



III

where the groups R^5 are the same or different and each is hydrogen or C_{1-4} alkyl, and m is from 1 to 4, in which preferably the groups R^5 are the same preferably methyl.

In phosphobetaine based groups, X may have the general formula IV



IV

in which A^5 is a valence bond, -O-, -S- or -NH-, preferably -O-;

R^6 is a valence bond (together with A^5) or alkanediyl, -C(O)alkylene- or -C(O)NH alkylene preferably alkanediyl, and preferably containing from 1 to 6 carbon atoms in the alkanediyl chain;

W^2 is S, PR^7 or NR^7;

the or each group R^7 is hydrogen or alkyl of 1 to 4 carbon atoms or the two groups R^7 together with the heteroatom to which they are attached form a heterocyclic ring of 5 to 7 atoms;

R^8 is alkanediyl of 1 to 20, preferably 1 to 10, more preferably 1 to 6 carbon atoms;

A^6 is a bond, NH, S or O, preferably O; and

R^9 is a hydroxyl, C_{1-12} alkyl, C_{1-12} alkoxy, C_{7-18} aralkyl, C_{7-18} -aralkoxy, C_{6-18} aryl or C_{6-18} aryloxy group.

Monomers comprising a group of the general formula IV may be made by methods as described in JP-B-03-031718, in which an amino substituted monomer is reacted with a phospholane.

In compounds comprising a group of the general formula IV, it is preferred that

A^5 is a bond;

R^6 is a C_{2-6} alkanediyl;

W^2 is NR^7:

each R^7 is C_{1-4} alkyl;

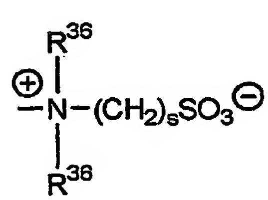
R^8 is C_{2-6} alkanediyl;

A^6 is O; and

R^9 is C_{1-4} alkoxy.

Alternatively X may be a zwitterion in which the anion comprises a sulphate, sulphonate or carboxylate group.

One example of such a group is a sulphobetaine group, of the general formula XI



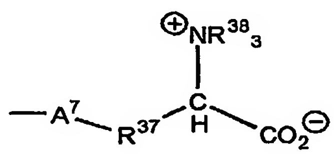
XI

where the groups R^{36} are the same or different and each is hydrogen or C_{1-4} alkyl and s is from 2 to 4.

Preferably the groups R^{36} are the same. It is also preferable that at least one of the groups R^{36} is methyl, and more preferable that the groups R^{36} are both methyl.

Preferably s is 2 or 3, more preferably 3.

Another example of a zwitterionic group having a carboxylate group is an amino acid moiety in which the alpha carbon atom (to which an amine group and the carboxylic acid group are attached) is joined through a linker group to the backbone of the biocompatible polymer. Such groups may be represented by the general formula XII



XII

in which A^7 is a valence bond, -O-, -S- or -NH-, preferably -O-,

R^{37} is a valence bond (optionally together with A^7) or alkanediyl, -C(O)alkylene- or -C(O)NHalkylene, preferably alkanediyl and preferably containing from 1 to 6 carbon atoms; and

the groups R^{38} are the same or different and each is hydrogen or alkyl of 1 to 4 carbon atoms, preferably methyl, or two or three of the groups R^{38}, together with the nitrogen to which they are attached, form a heterocyclic ring of from 5 to 7 atoms, or the three group R^{38} together with the nitrogen atom to which they are attached form a fused ring heterocyclic structure containing from 5 to 7 atoms in each ring.

Another example of a zwitterion having a carboxylate group is a carboxy betaine $-N^{\oplus}(R^{39})_2(CH_2)_rCOO^{\ominus}$ in which the R^{39} groups are the same or different and each is hydrogen or R_{1-4} alkyl and r is 2 to 6, preferably 2 or 3.

In the zwitterionic monomer of the general formula I it is preferred that the ethylenic unsaturated group Y is $H_2C=CR-CO-A-$. Such acrylic moieties are preferably methacrylic, that is in which R is methyl, or acrylic, in which R is hydrogen. Whilst the compounds may be (meth)acrylamido compounds

(in which A is NR^1), in which case R^1 is preferably hydrogen, or less preferably, methyl, most preferably the compounds are esters, that is in which A is O.

In monomers of the general formula I, especially where Y is the preferred (alk)acrylic group, B is most preferably an alkanediyl group. Whilst some of the hydrogen atoms of such group may be substituted by fluorine atoms, preferably B is an unsubstituted alkanediyl group, most preferably a straight chain group having 2 to 6 carbon atoms.

A particularly preferred zwitterionic monomer is 2-methacryloyloxyethyl-2'-trimethylammonium ethyl phosphate inner salt.

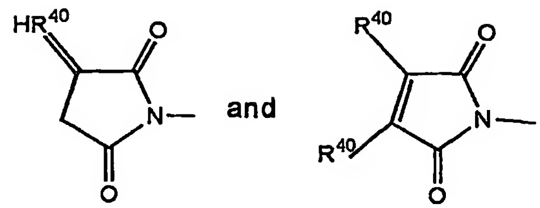
The ionic block may be formed of condensation polymers, such as polyethers, polyesters, polyamides, polyanhydrides polyurethanes, polyethers, polyimines, polypeptides, polyureas, polyacetals, polysaccharides or polysiloxanes. One example of a suitable ionic block is polyethyleneimine, copolymers of which with polyalkylene oxides have been investigated as drug delivery components. Preferably, however, the block is formed by radical polymerisation of ethylenically unsaturated monomers.

It is preferred that the ionic block comprise pendant cationic or anionic groups. Cationic pendant groups are, for instance, primary, secondary or tertiary amines, capable of being protonated at pH's in the range 4 to 10. Alternatively a cationic group may be a phosphine. An anionic group may be a phosphate, phosphonate, sulphate, sulphonate, carbonate or preferably carboxylate group.

Suitable ionic monomers from which the ionic block is formed have the general formula VI

$$Y^1B^1Q \qquad\qquad VI$$

in which Y^1 is selected from $H_2C{=}CR^{40}\text{-}CO\text{-}A^8\text{-}$, $H_2C{=}CR^{41}\text{-}C_6H_4\text{-}A^9\text{-}$, $H_2C{=}CR^{40}\text{-}CH_2A^{10}$, $R^{42}O\text{-}CO\text{-}CR^{40}{=}CR^{40}\text{-}CO\text{-}O$, $R^{40}CH{=}CH\text{-}CO\text{-}O\text{-}$, $R^{40}CH{=}C(COOR^{42})CH_2\text{-}CO\text{-}O$,



A^8 is -O- or NR^{41};

A^9 is selected from a bond, $(CH_2)_qA^{10}$ and $(CH_2)_q$ SO_3- in which q is 1 to 12;

A^{10} is selected from a bond, -O-, -O-CO-, -CO-O, -CO-NR^{41}-, -NR^{41}CO-, -O-CO-NR^{41}-, -NR^{41}-CO-O-;

R^{40} is hydrogen or C_{1-4} alkyl;

R^{41} is hydrogen, C_{1-4} alkyl or B^1Q;

R^{42} is hydrogen or C_{1-4} alkyl;

B^1 is a bond, or a straight branched alkanediyl, alkylene oxaalkylene, or alkylene (oligooxalkylene) group, optionally containing one or more fluorine substituents; and

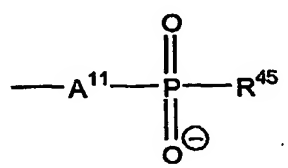
Q is an ionic or ionisable moiety.

By the term ionic monomer, we include ionisable monomers.

Examples of cationic or cationisable groups Q have the formula -NR^{43}_p, -PR^{43}_p and SR^{43}_r, in which p is 2 or 3, r is 1 or 2, the groups R^{43} are the same or different and each is selected from the group consisting of hydrogen, C_{1-24} alkyl and aryl, or two of the groups R^{43} together with the heteroatom to which they are attached from a 5 to 7 membered heterocyclic ring or three R^{43} groups together with the heteroatom to which they are attached form a 5 to 7 membered heteroaromatic ring, either of which rings may be fused to another 5 to 7 membered saturated or unsaturated ring, and any of the R^{43} groups may be substituted by amino or hydroxyl gruops or halogen atoms.

Preferably Q is NR^{43}_2 where R^{43} is C_{1-12}-alkyl. Preferably both R^{43}'s are the same. Particularly useful results have been achieved where the groups R^{43} are C_{1-4} alkyl, especially ethyl.

Where the monomer of the general formula V provides anionic or anionisable groups, for instance carboxylate, or carboxylic acid group, B^1 is a bond, A^8 is -O- and Q is hydrogen. Alternative monomers providing carboxylate or carboxylic acid moieties have B^1 as other than a bond, and Q as a carboxylate or carboxylic acid group. Where Q is an anionic or anionisable group other than carboxylate or carboxylic acid group, then B^1 is other than a bond, and Q is a group of general formula VII



VII

in which A^{11} is a bond, MH, S or O, preferably O; and

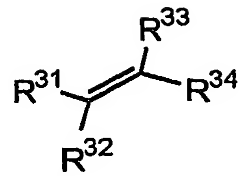
R^{45} is a hydroxyl, C_{1-12}-alkyl, C_{1-12}-alkoxy, C_{7-18}-aralkyl, C_{7-18}-arylkoxy, C_{6-18}-aryl or C_{6-18}-aryloxy group.

Alternatively Q may be a group SO_3^-.

B^1 is preferably C_{2-6}-alkanediyl, preferably $(CH_2)_{2-6}$.

Either or both the zwitterionic and ionic blocks may include comonomers, for instance to provide functionality, control over hydrophobicity, control over pH sensitivity, pK_A or pK_B as the case may be, or as general diluents. For instance comonomers providing functionality may be useful to provide conjugation of pendant groups following polymerisation and/or micelle formation, to targeting moieties, or to provide for conjugation between the biologically active molecule and the polymer. Alternatively, functional groups may allow for crosslinking of the polymer following micelle formation, to confer increased stability on the micellar structure.

Examples of suitable comonomers are compounds of the general formula X



X

in which R^{31} is selected from hydrogen, halogen, C_{1-4} alkyl and groups $COOR^2$ in which R^2 is hydrogen and C_{1-4} alkyl;

R^{32} is selected from hydrogen, halogen and C_{1-4} alkyl;

R^{33} is selected from hydrogen, halogen, C_{1-4} alkyl and groups $COOR^2$ provided that R^{31} and R^{33} are not both $COOR^2$; and

R^{34} is a C_{1-10} alkyl, a C_{1-20} alkoxycarbonyl, a mono-or di-(C_{1-20} alkyl) amino carbonyl, a C_{6-20} aryl (including alkaryl) a C_{7-20} aralkyl, a C_{6-20} aryloxycarbonyl, a C_{1-20} -aralkyloxycarbonyl, a C_{6-20} arylamino carbonyl, a C_{7-20} aralkyl-amino, a hydroxyl or a C_{2-10} acyloxy group, any of which may have one or more substituents selected from halogen atoms, alkoxy, oligo-alkoxy, aryloxy, acyloxy, acylamino, amine (including mono and di-alkyl amino and trialkylammonium in which the alkyl groups may be substituted), carboxyl, sulphonyl, phosphoryl, phosphino, (including mono- and di- alkyl phosphine and tri-alkylphosphonium), zwitterionic, hydroxyl groups, vinyloxycarbonyl and other vinylic or allylic substituents, and reactive silyl or silyloxy groups, such as trialkoxysilyl groups;

or R^{34} and R^{33} or R^{34} and R^{32} may together form $-CONR^{35}CO$ in which R^{35} is a C_{1-20} alkyl group.

It is preferred for at least two of the groups $R^{31}R^{32,}R^{33}$ and R^{34} to be halogen or, more preferably, hydrogen atoms. Preferably R^{31} and R^{32} are both hydrogen atoms. It is particularly preferred that compound of general formula X be a styrene-based or acrylic based compound. In styrene based compounds R^{34} represents an aryl group, especially a substituted aryl group in which the substituent is an amino alkyl group, a carboxylate or a sulphonate group. Where the comonomer is an acrylic type compound, R^{34} is an alkoxycarbonyl, an alkyl amino carbonyl, or an aryloxy carbonyl group. Most preferably in such compounds R^{34} is a C_{1-20} -alkoxy carbonyl group,

optionally having a hydroxy substituent. Acrylic compounds are generally methacrylic in which case R^{33} is methyl.

Preferably the comonomer is a non-ionic comonomer, such as a C_{1-24} alkyl(alk)-acrylate or -acrylamide, mono- or di- hydroxy-C_{1-6}-alkyl(alk)-acrylate, or -acrylamide, oligo(C_{2-3} alkoxy) C_{2-18}-alkyl (alk)-acrylate, or -acrylamide, styrene, vinylacetate or N-vinyllactam.

The block copolymer may be a simple A-B block copolymer, or may be an A-B-A or B-A-B block copolymer (where A is the zwitterionic block and B is the ionic block). It may also be an A-B-C, A-C-B or B-A-C block copolymer, where C is a different type of block. C blocks may, for instance, comprise functional, e.g. cross-linking or ionic groups, to allow for reactions of the copolymer, for instance in the novel compositions. Crosslinking reactions especially of A-C-B type copolymers, may confer useful stability on drug-containing micelles. Cross-linking may be covalent, or sometimes, electrostatic in nature. Cross-linking may involve addition of a separate reagent to link functional groups, such as using a difunctional alkylating agent to link two amino groups.

The block copolymers preferably have controlled molecular weights. It is preferable for each of the blocks to have molecular weight controlled within a narrow band, that is to have a narrow polydispersity. The polydispersity of molecular weight should, for instance, be less than 2.0, more preferably less than 1.5, for instance in the range 1.1 to 1.4.

The degree of polymerisation of an ionic block is in the range 5 to 2000, preferably 10 to 500, more preferably 10 to 250. A zwitterionic block has a degree of polymerisation in the range 2 to 1000, preferably 5 to 250 more preferably 10 to 100. Generally the relative lengths of the ionic to zwitterionic blocks is in the range 1:5 to 10:1, preferably 1:1 to 5:1.

It may be possible to synthesise the block copolymer by initial formation of a low poly dispersity, low molecular weight initial block using control of initiator and chain transfer agent (which permanently terminates chain formation), with the initial block then being derivatised to act as a

suitable radical initiator in a subsequent block forming step, by the technique described by Inoue *et al* J. Contr. Rel. 1998, 51, 221-229. Preferably, however, the polymerisation of at least one of the blocks is by controlled radical polymerisation for instance a living radical polymerisation process.

A living radical polymerisation process may be a group transfer radical polymerisation, for instance in which an N→O, or other carbon-, sulphur-, and oxygen- centered radical group is transferred from an initiator compound to a monomer. Preferably, however, the process is an atom transfer radical polymerisation process. Preferably such a process is used to form each block of the block copolymer.

In the atom or group transfer radical polymerisation process, the initiator has a radically transferable atom or group, and the catalyst comprises a transition metal compound and a ligand, in which the transition metal compound is capable of participating in a redox cycle with the initiator and dormant polymer chain, and the ligand is either any N-, O-, P- or S- containing compound which can coordinate with the transition metal atom in a σ-bond, or any carbon-containing compound which can coordinate with the transition metal in a π-bond, such that direct bonds between the transition metal and growing polymer radicals and not formed.

Preferably the radical initiator is of the general formula V

$$R^{11}R^{12}R^{13}C\text{-}X^2 \qquad \text{V}$$

where:

X^2 is selected from the group consisting of Cl, Br, I, OR^{10}, SR^{14}, SeR^{14}, $OP(=O)R^{14}$, $OP(=O)(OR^{14})_2$, $O\text{-}N(R^{14})_2$ and $S\text{-}C(=S)N(R^{14})_2$, where R^{10} is alkyl of from 1 to 20 carbon atoms in which each of the hydrogen atoms may be independently replaced by halide, R^{14} is aryl or a straight or branched C_1-C_{20} alkyl group, and where an $N(R^{14})_2$ group is present, the two R^{14} groups may be joined to form a 5- or 6-membered heterocyclic ring; and

R^{11}, R^{12} and R^{13} are each independently selected from the group consisting of H, halogen, C_1-C_{20} alkyl, C_3-C_8 cycloalkyl, $C(=O)R^{15}$,

$C(=O)NR^{16}R^{17}$, COCl, OH, CN, C_2-C_{20} alkenyl, C_2-C_{20} alkenyl oxiranyl, glycidyl, aryl, heterocyclyl, aralkyl, aralkenyl, C_1-C_6 alkyl in which from 1 to all of the hydrogen atoms are replaced with halogen, C_1-C_6 alkyl substituted with from 1 to 3 substituents selected from the group consisting of C_1-C_4 alkoxy, aryl, heterocyclyl, $C(=O)R^{15}$, $C(=O)NR^{16}R^{17}$, $-CR^{12}R^{13}X^2$, oxiranyl and glycidyl;

where R^{15} is alkyl of from 1 to 20 carbon atoms, alkoxy of from 1 to 20 carbon atoms, oligo(alkoxy) in which each alkoxy group has 1 to 3 carbon atoms, aryloxy or heterocyclyloxy any of which groups may have substituents selected from optionally substituted alkoxy, oligoalkoxy, amino (including mono- and di-alkyl amino and trialkyl ammonium, which alkyl groups, in turn may have substituents selected from acyl, alkoxycarbonyl, alkenoxycarbonyl, aryl and hydroxy) and hydroxyl groups; and

R^{16} and R^{17} are independently H or alkyl of from 1 to 20 carbon atoms which alkyl groups, in turn may have substituents selected from acyl, alkoxycarbonyl, alkenoxycarbonyl, aryl and hydroxy, or R^{16} and R^{17} may be joined together to form an alkanediyl group of from 2 to 5 carbon atoms, thus forming a 3- to 6-membered ring;

such that not more than two of R^{11}, R^{12} and R^{13} are H.

In the initiator of the general formula V it is preferred that no more than one of R^{11}, R^{12} and R^{13}, and preferably none, is hydrogen. Suitably at least one, and preferably both of R^{11} and R^{12} is methyl. R^{13} is suitably a group CO-R^{15} in which R^{15} is preferably alkoxy of from 1 to 20 carbon atoms, oligo(alkoxy) in which each alkoxy group has 1 to 3 carbon atoms, aryloxy or heterocyclyloxy any of which groups may have substituents selected from optionally substituted alkoxy, oligoalkoxy, amino (including mono-- and di-alkyl amino and trialkyl ammonium, which alkyl groups, in turn may have substituents selected from acyl, alkoxycarbonyl, alkenoxycarbonyl, aryl and hydroxy) and hydroxyl groups.

Since any of R^{11}, R^{12} and R^{13} may comprise a substituent $C^{12}R^{13}X^2$, the initiator may be di-, oligo- or poly- functional.

Selection of a suitable initiator is based on various considerations. Where the polymerisation is carried out in the liquid phase, in which the monomers are dissolved, it is preferable for the initiator to be soluble in that liquid phase. The initiator is thus selected for its solubility characteristics according to the solvent system which in turn is selected according to the monomers being polymerised.

Water-soluble initiators include, for instance the reaction product of monomethoxy-capped oligo(ethylene oxide) with 2-bromoisobutyryl bromide (OEGBr), 4-bromo-α-toluic acid or ethyl 2-bromopropanoic acid or 2-(N,N-dimethylamino) ethyl-2'-bromoisobutyrate.

The portion of the initiator $-C-R^{11}R^{12}R^{13}$ becomes joined to the first monomer of the growing polymer chain. The group X^2 becomes joined to the terminal unit of the polymer chain. Selection of a suitable initiator is determined in part by whether a terminal group having particular characteristics is required for subsequent functionality. The residue of the initiator at one or other end of the polymer may be reacted with biologically active moieties, such as targetting groups. Alternatively the initiator itself may comprise a group conferring ueful targetting or other useful properties without further reaction.

In an atom or group radical transfer polymerisation process the transition metal compound which comprises a component of the catalyst is $M_t^{n+}X'_n$, where:

M_t^{n+} may be selected from the group consisting of Cu^{1+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Ru^{2+}, Ru^{3+},Cr^{2+}, Cr^{3+}, Mo^{2+}, Mo^{3+}, W^{2+}, W^{3+}, Mn^{2+}, Mn^{3+}, Mn^{4+}, Rh^{3+}, Rh^{4+}, Re^{2+}, Re^{3+}, Co^{+}, Co^{2+}, Co^{3+}, V^{2+}, V^{3+}, Zn^{+}, Zn^{2+}, Ni^{2+}, Ni^{3+}, Au^{+}, Au^{2+}, Ag^{+} and Ag^{2+};

X' is selected from the group consisting of halogen, C_1-C_6-alkoxy, $(SO_4)_{1/2}$, $(PO_4)_{1/3}$, $(R^{18}PO_4)_{1/2}$, $(R^{18}{}_2PO_4)$, triflate, hexafluorophosphate, methanesulphonate, arylsulphonate, CN and $R^{19}CO_2$, where R^{18} is aryl or a straight or branched C_{1-20} alkyl and R^{19} is H or a straight or branched C_1-C_6 alkyl group which may be substituted from 1 to 5 times with a halogen; and

n is the formal charge on the metal ($0 \leq n \leq 7$).

Preferably X' is halide, most preferably chloride or bromide. Particularly suitable transition metal compounds are based on copper or ruthenium, for instance CuCl or $RuCl_2$.

In the catalyst, the ligand is preferably selected from the group consisting of:

a) compounds of the formulas:

$$R^{20}\text{-}Z\text{-}R^{21} \quad \text{and}$$

$$R^{20}\text{-}Z\text{-}(R^{22}\text{-}Z)_m\text{-}R^{21}$$

where:

R^{20} and R^{21} are independently selected from the group consisting of H, C_1-C_{20} alkyl, aryl, heterocyclyl and C_1-C_6 alkoxy, C_1-C_4 dialkylamino, $C(=O)R^{22}$, $C(=O)R^{23}R^{24}$ and $A^7C(=O)R^{25}$, where A^7 may be NR^{26} or O; R^{22} is alkyl of from 1 to 20 carbon atoms, aryloxy or heterocyclyloxy; R^{23} and R^{24} are independently H or alkyl of from 1 to 20 carbon atoms or R^{23} and R^{24} may be joined together to form an alkanediyl group of from 2 to 5 carbon atoms, thus forming a 3- to 6-membered ring; R^{25} is H, straight or branched C_1-C_{20} alkyl or aryl and R^{26} is hydrogen, straight or branched; $C_{1\text{-}20}$-alkyl or aryl; or R^{20} and R^{21} may be joined to form, together with Z, a saturated or unsaturated ring;

Z is O, S, NR^{27} or PR^{27}, where R^{27} is selected from the same group as R^{20} and R^{21}, and where Z is PR^{27}, R^{27} can also C_1-C_{20} alkoxy or Z may be a bond, CH_2 or a fused ring, where one or both of R^{20} and R^{21} is heterocyclyl,

each R^{22} is independently a divalent group selected from the group consisting of C_1-C_8 cycloalkanediyl, C_1-C_8 cycloalkenediyl, arenediyl and heterocyclylene where the covalent bonds to each Z are at vicinal positions or R^{22} may be joined to one or both of R^{20} and R^{21} to formulate a heterocyclic ring system; and

m is from 1 to 6;

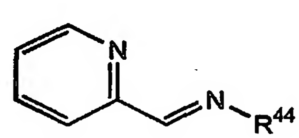
b) CO;

c) porphyrins and porphycenes, which may be substituted with from 1 to 6 halogen atoms, C_{1-6} alkyl groups, C_{1-6}-alkoxy groups, C_{1-6} alkoxycarbonyl, aryl groups, heterocyclyl groups, and C_{1-6} alkyl groups further substituted with from 1 to 3 halogens;

d) compounds of the formula $R^{23}R^{24}C(C(=O)R^{25})_2$, where R^{25} is C_{1-20} alkyl, C_{1-20} alkoxy, aryloxy or heterocyclyloxy; and each of R^{23} and R^{24} is independently selected from the group consisting of H, halogen, C_{1-20} alkyl, aryl and heterocyclyl, and R^{23} and R^{24} may be joined to form a C_{1-8} cycloalkyl ring or a hydrogenated aromatic or heterocyclic ring, of which the ring atoms may be further substituted with 1 to 5 C_{1-6} alkyl groups, C_{1-6} alkoxy groups, halogen atoms, aryl groups, or combinations thereof; and

e) arenes and cyclopentadienyl ligands, where said cyclopentadienyl ligand may be substituted with from one to five methyl groups, or may be linked through and ethylene or propylene chain to a second cyclopentadienyl ligand.

Selection of a suitable ligand is, for instance, based upon the solubility characteristics and/or the separability of the catalyst from the product polymer mixture. Generally it is catalyst to be soluble in a liquid reaction mixture, although under some circumstances it may be possible to immobilise the catalyst, for instance an a porous substrate. For the preferred process, which is carried out in the liquid phase, the ligand is soluble in a liquid phase. The ligand is generally a nitrogen containing ligand. The preferred ligand may be a compound including a pyridyl group and an imino moiety, such as bipyridine, or



where R^{44} is a suitable alkyl group, the substituent being variable and adaptable to confer desired solubility characteristics or may be triphenylphosphine or 1,1,4,7,10,10-hexamethyl-triethylene tetramine.

Such ligands are usefully used in combination with copper chloride and ruthenium chloride transition metal compounds as part of the catalyst.

A living radical polymerisation process is preferably carried out to achieve a degree of polymerisation in the or each block in the range 5 to 500. Preferably the degree of polymerisation is in the range 10 to 100, more preferably in the range 10 to 50. In the preferred group or atom transfer radical polymerisation technique, the degree of polymerisation is directly related to the initial ratios of initiator to monomer. Preferably the ratio is in the range 1:(5 to 500), more preferably in the range of 1:(10 to 100), most preferably in the range 1:(10 to 50).

The ratio of metal compound and ligand in the catalyst should be approximately stoichiometric, based on the ratios of the components when the metal ion is fully complexed. The ratio should preferably be in the range 1:(0.5 to 2) more preferably in the range 1:(0.8:1.25). Preferably the range is about 1:1.

In the living radical polymerisation process, the catalyst may be used in amounts such that a molar equivalent quantity as compared to the level of initiator is present. However, since catalyst is not consumed in the reaction, it is generally not essential to include levels of catalyst as high as of initiator. The ratio of catalyst (based on transition metal compound) to initiator is preferably in the range 1:(1 to 50), more preferably in the range 1:(1 to 10).

Whilst the polymerisation reaction may be carried out in the gaseous phase, it is more preferably carried out in the liquid phase. The reaction may be heterogeneous, that is comprising a solid and a liquid phase, but is more preferably homogeneous. Preferably the polymerisation is carried out in a single liquid phase. Where the monomer is liquid, it is sometimes unnecessary to include a non-polymerisable solvent. More often, however, the polymerisation takes place in the presence of a non-polymerisable

solvent. The solvent should be selected having regard to the nature of the zwitterionic monomer and any comonomer, for instance for its suitability for providing a common solution containing both monomers. The solvent may comprise a single compound or a mixture of compounds.

It has been found that, especially where the zwitterionic monomer is MPC, that it is desirable to include water in the polymerisation mixture. Preferably water should be present in an amount in the range 10 to 100% by weight based on the weight of ethylenically unsaturated monomer. Preferably the total non-polymerisable solvent comprised 1 to 500% by weight based on the weight of ethylenically unsaturated monomer. It has been found that the zwitterionic monomer and water should be in contact with each other for as short a period as possible prior to contact with the initiator and catalyst. It may be desirable therefore for all the components of the polymerisation other than the zwitterionic monomer to be premixed and for the zwitterionic monomer to be added to the premix as the last additive.

It is often desired to copolymerise MPC or other zwitterionic monomer with a comonomer which is insoluble in water. In such circumstances, a solvent or co-solvent (in conjunction with water) is included to confer solubility on both MPC and the more hydrophobic monomer. Suitable organic solvents are ethers, esters and, most preferably, alcohols. Especially where a mixture of organic solvent and water is to used, suitable alcohols are C_{1-4} -alkanols. Methanol is found to be particularly suitable in the polymerisation process of the invention.

The process may be carried out at raised temperature, for instance up to 60 to 80 °C. However it has been found that the process proceeds sufficiently fast at ambient temperature.

The living radical polymerisation process has been found to provide polymers of zwitterionic monomers having a polydispersity (of molecular weight) of less than 1.5, as judged by gel permeation chromatography. Polydispersities in the range 1.2 to 1.4 for the or each block are preferred.

In the composition the relative amounts of biologically active compound and of polymer may be about stoichiometric in terms of the counterionic groups. Alternatively there may be an excess of one charge over the other for instance up to 5 or 10 times excess. The level may depend on stability factors or on interactions of the components of the composition with biological systems. For instance, Rungfardthong, *et al*, *op. cit.*, show that the level of excess catioinc polymer over DNA may affect transfection levels. Appropriate levels of the biologically active compound and polymer may be determined by experimentation. The particles may be analysed for their ζ potential. This technique determines the presence of overall charge on the surface of particles. Stability and activity may be determined by available assays. ζ potential is determined by agarose gel electro phoresis. It has been found that the presence of zwitterionic groups in the aqueous composition stabilises the dispersed particles, without requiring addition of stabilisers, or using an excess of drug or polymer. The compositions may additionally contain buffers or other salts or pH-modifying components, stabilisers etc.

Further probes into the particles in the composition may be by dynamic light scattering investigations, which may give a value for average aggregate diameters. Small angle neutron scattering may also be used to provide structural details inside the particles, as may electron microscopy, for instance transmission electron microscopy (TEM).

It is believed that the compositions will be particularly useful for allowing gene or ODN delivery into cells. Thus the compositions may be useful for administering to the patients in need of therapy by the biologically active molecule. The compositions may thus be suitable for administration IV, IP or IM for instance. The effect of the compositions on intracellular delivery may be illustrated using *in vitro* test systems. For instance delivery of genes into cells may be determined by using, as the biologically active molecule, a plasmid encoding a model gene, the product of which may be observed. Suitable vectors are available encoding luciferase and/or β-

galactosidase. Such tests may be carried out in conjunction with cell proliferation assays using tritium-labelled thymidine and using as positive controls known cationic polymer delivery systems such as poly-L-lysine, or PEO-PEI block copolymers.

Cytotoxicity determinations may be conducted. Such tests may, for instance, be useful to determine the base toxicity of the polymers themselves. Alternatively, where the drug to be delivered is intended to be cytotoxic, a cytotoxicity test may reveal the success of the drug delivery system. Stability of the compositions may be investigated, for instance by contacting the compositions with pH modifiers, salts and/or serum and determining physical and biologically stability. Resistance to degradation by enzymic reactions, such as attack of nucleic acid by nucleases is relevant to the utility of the invention in formulating nucleic acids, as is the effect on ethidium bromide intercalation.

The following examples illustrate the invention.

Example 1

A-B block copolymers were formed by an atom transfer polymerisation with MPC being homopolymerised in a first block forming step using an oligo(ethylene glycol) initiator as described by Ashford E.J. *et al* in Chem. Commun. 1999, 1285 (the reaction product of monomethoxy-capped oligo(ethylene glycol) and 2-bromoisobutyryl bromido) in the presence of bipyridine ligand and copper (I) bromide catalyst. DEA (diethylaminoethyl methacrylate) was polymerised in a second block forming step. The degree of polymerisation for each block is indicated in Table 1 showing the results.

The reaction conditions were [MPC] = 2.02M (6.0g in 10ml methanol), [MPC]: [OEG-Br]:[CuBr]:[bipy] = (30 or 20 as shown in Table 1):1:1:2, T = 20°C; MPC was polymerised first in all cases followed by addition and polymerisation of an appropriate amount of neat DEA. Almost complete monomer conversion was achieved after the time indicated in Table 1 for the diblock, as indicated by ^{1}H NMR spectroscopy (no residual vinyl double bonds). The reaction mixture was diluted with methanol and passed through

a silica column to remove residual ATRP catalyst. After solvent evaporation, the products were dried under vacuum at room temperature.

Table 1: Data of the polymerization of MPC – DEAEMA diblock copolymers in methanol

Ex #	Comonomer	MPC in copolymer (mol %)	TargetDp	[Amine] (M)	Time for > 99% Conversion		Mn (AGPC)		Mw / Mn	
					MPC HOMO (mins)	MPC Diblock (h)	MPC(a) HOMO	MPC(b) Diblock	MPC HOMO	MPC Diblock
1	DEA	50	20:20	1.35	180	20	6200	14000	1.15	1.22
2	DEA	33	10:20	1.35	180	21	3500	11000	1.17	1.29
3	DEA	50	30:30	2.02	130	20	10000	21000	1.18	1.30
4	DEA	33	30:60	4.04	130	22	11000	31000	1.19	1.29
5	DEA	23	30:100	6.73	130	23	11000	43000	1.19	1.28

DEA = diethylaminoethylmethacrylate

AGPC = aqueous gel permeation chromatography

The MPC-DEA block copolymers were dissolved in McIlvaines buffer at a concentration of 1mM and at pH 4. The pH was then adjusted upwards with NaOH to pH 8, and 10.8, so the micelles would form. A series of half dilutions were then prepared from the micellised polymers, using McIlvaines buffer of the same pH as the polymer solution.

To demonstrate the polymer shift from unimer to micelle state in response to pH increase, a control of polymer at pH 4 was carried using the same technique and conditions as that used for the pH8 and pH10.8 samples using the 30:60 MPC:DEA block copolymer. The polymer solutions at pH4 and pH8 were also analysed using photon correlation spectroscopy (PCS) to measure the hydrodynamic diameter of the particles based on the intensity of scattered light, and calculated using the Stokes-Einstein equation, as described in ISO13321 British Standards Institution. 1997, BS3046: Part 8: 1997: ISO 13321: "Photon correlation spectroscopy", in Methods for determination of particle size distribution, BSI publications, Chiswick, UK, p 1-21, with subsequent analysis and determination of intensity size distributions using the CONTIN algorithm. Measurement was carried out using a Malvern Zetasizer 3000HS, using a 10mW He-Ne laser, with a wavelength of 633nm, and a high sensitivity avalanche photodiode detector fixed at a 90 degree angle to the laser, at a temperature of 25°C. Samples were sonicated for 5 minutes and filtered through a 0.2 micron filter prior to measurement, to remove any aggregation and possible dust contamination.

In Figure 1 the shift from unimer to micelle in response to increased pH can be seen. At pH4 (grey shaded curve) only unimers with a mean diameter of 11.3nm are present, however when the pH is raised to pH8 (black shaded curve) there is a clear increase in mean diameter from 11.3nm up to 37.5nm, indicating the unimers have undergone micellar self assembly in response to increased pH.

Example 2

Complexes of the preformed micelles formed in Example 1 with DNA are produced by mixing aqueous solution of polymer with aqueous solutions of DNA (e.g. calf thymus DNA, plasmid DNA encoding chloramphenicol acetyl transferases reporter gene, and plasmid DNA encoding a luciferase reporter gene) 1:5 to 10:1 approximately at various ratios in the range (cationic equivalents to anionic equivalents). The resultant compositions were then observed using any or all of the following techniques as described by Rungfordthong *et al op.cit*:

agarose gel electrophoresis using Coomassie blue dye to develop,

ethidium bromide displacement assay (using calf thymus DNA), scattering intensity studies including in the presence of salts and at different pH using a Malvern PCS system (to reveal particles in suspension and measure size, as described for the polymer micelles in Example 1) and transfection studies e.g. using the luciferase encoding plasmid in A549 or other cell culture.

Controls may be based on naked DNA and comparisons with DNA-transfection systems based on cationic polymers such as poly-L-lysine, cationic dendrimers or block copolymers of PEG and cationic monomers.

CLAIMS

1. A liquid aqueous composition comprising a suspension of a polymer having an overall ionic charge and a biologically active compound having a charge opposite that of the polymer and is characterised in that the polymer has pendant zwitterionic groups.

2. A composition according to claim 1 in which the biologically active compound is anionic, preferably polyanionic.

3. A composition according to claim 2 in which where the active compound is a nucleic acid.

4. A composition according to claim 3 in which the nucleic acid is selected from oligo nucleotides, having 5 to 80 bases, single stranded RNA, single stranded DNA and double stranded DNA, preferably plasmid DNA.

5. A composition according to claim 1 in which the biologically active compound is an anionic drug.

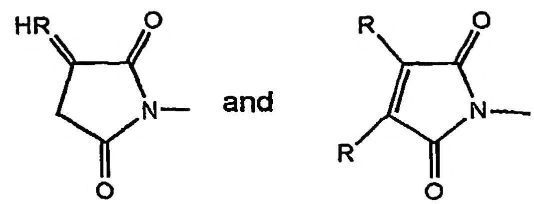
6. A composition according to any preceding claim in which the biologically active compound and polymer are associated with one another in the form of particles having an average diameter less than 200 μm.

7. A composition according to any preceding claim in which the polymer is a block copolymer comprising a zwitterionic block comprising the said pendant zwitterionic groups, and an ionic block, comprising the said ionic groups.

8. A composition according to claim 7 in which the zwitterionic block is formed from ethylenically unsaturated monomers including a zwitterionic monomer having the general formula

$$YBX \qquad I$$

in which Y is an ethylenically unsaturated group selected from $H_2C{=}CR\text{-}CO\text{-}A\text{-}$, $H_2C{=}CR\text{-}C_6H_4\text{-}A^1\text{-}$, $H_2C{=}CR\text{-}CH_2A^2$, $R^2O\text{-}CO\text{-}CR{=}CR\text{-}CO\text{-}O$, $RCH{=}CH\text{-}CO\text{-}O\text{-}$, $RCH{=}C(COOR^2)CH_2\text{-}CO\text{-}O$,



A is -O- or NR^1;

A^1 is selected from a bond, $(CH_2)_lA^2$ and $(CH_2)_l$ SO_3- in which l is 1 to 12;

A^2 is selected from a bond, -O-, O-CO-, CO-O, CO-NR^1-, -NR^1-CO, O-CO-NR^1-, NR^1-CO-O-;

R is hydrogen or C_{1-4} alkyl;

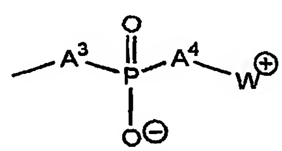
R^1 is hydrogen, C_{1-4} alkyl or BX;

R^2 is hydrogen or C_{1-4} alkyl;

B is a bond, or a straight branched alkanediyl, alkylene oxaalkylene, or alkylene (oligooxalkylene) group, optionally containing one or more fluorine substituents;

X is a zwitterionic group.

9. A composition according to claim 8 in which X is an ammonium, phosphonium, or sulphonium phosphate or phosphonate ester zwitterionic group, more preferably a group of the general formula II-



II

in which the moieties A^3 and A^4, which are the same or different, are -O-, -S-, -NH- or a valence bond, preferably -O-, and W^+ is a group comprising an ammonium, phosphonium or sulphonium cationic group and a group linking the anionic and cationic moieties which is preferably a C_{1-12}-alkanediyl group,

preferably in which W^+ is a group of formula

-W^1-$N^+R^3_3$, -W^1-$P^+R^4_3$, -W^1-$S^+R^4_2$ or -W^1-Het^+ in which:

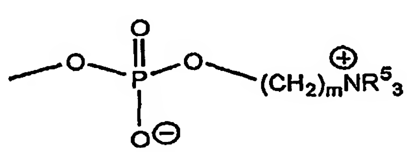
W^1 is alkanediyl of 1 or more, preferably 2-6 carbon atoms optionally containing one or more ethylenically unsaturated double or triple bonds, disubstituted-aryl (arylene), alkylene arylene, arylene alkylene, or alkylene aryl alkylene, cycloalkanediyl, alkylene cycloalkyl, cycloalkyl alkylene or alkylene cycloalkyl alkylene, which group W^1 optionally contains one or more fluorine substituents and/or one or more functional groups; and

either the groups R^3 are the same or different and each is hydrogen or alkyl of 1 to 4 carbon atoms, preferably methyl, or aryl, such as phenyl, or two of the groups R^3 together with the nitrogen atom to which they are attached form an aliphatic heterocyclic ring containing from 5 to 7 atoms, or the three groups R^3 together with the nitrogen atom to which they are attached as heteroaromatic ring having 5 to 7 atoms, either of which rings may be fused with another saturated or unsaturated ring to form a fused ring structure containing from 5 to 7 atoms in each ring, and optionally one or more of the groups R^3 is substituted by a hydrophilic functional group, and

the groups R^4 are the same or different and each is R^3 or a group OR^3, where R^3 is as defined above; or

Het is an aromatic nitrogen-, phosphorus- or sulphur-, preferably nitrogen-, containing ring, for example pyridine.

10. A composition according to claim 8 in which X has the preferred general formula III



III

where the groups R^5 are the same or different and each is hydrogen or C_{1-4} alkyl, and m is from 1 to 4, in which preferably the groups R^5 are the same preferably methyl.

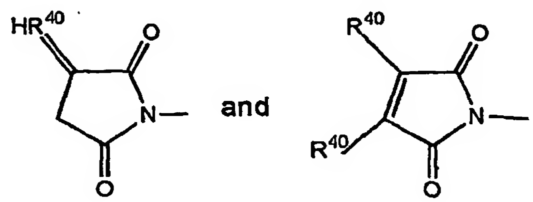
11. A composition according to any of claims 1 to 10 in which the ethylenic unsaturated group Y is $H_2C{=}CR\text{-}CO\text{-}A\text{-}$, in which R is preferably hydrogen or methyl and A is preferably NH or, more preferably, O.

12. A composition according to any of claims 7 to 11 in which the zwitterionic monomer is 2-methacryloyloxyethyl-2'-trimethylammonium ethyl phosphate inner salt.

13. A composition according to claim 7 in which the ionic block is formed of ethylenically unsaturated monomers including anionic monomer of general formula VI

$$Y^1B^1Q \qquad \text{VI}$$

in which Y^1 is selected from $H_2C{=}CR^{40}\text{-}CO\text{-}A^8\text{-}$, $H_2C{=}CR^{41}\text{-}C_6H_4\text{-}A^9\text{-}$, $H_2C{=}CR^{40}\text{-}CH_2A^{10}$, $R^{42}O\text{-}CO\text{-}CR^{40}{=}CR^{40}\text{-}CO\text{-}O$, $R^{40}CH{=}CH\text{-}CO\text{-}O\text{-}$, $R^{40}CH{=}C(COOR^{42})CH_2\text{-}CO\text{-}O$,



A^8 is -O- or NR^{41};

A^9 is selected from a bond, $(CH_2)_qA^{10}$ and $(CH_2)_q\ SO_3\text{-}$ in which q is 1 to 12;

A^{10} is selected from a bond, -O-, O-CO-, CO-O, CO-NR^{41}-, -NR^{41}-CO, O-CO-NR^{41}-, NR^{41}-CO-O-;

R^{40} is hydrogen or C_{1-4} alkyl;

R^{41} is hydrogen, C_{1-4} alkyl or B^1Q.

R^{42} is hydrogen or C_{1-4} alkyl;

B^1 is a bond, or a straight branched alkanediyl, alkylene oxaalkylene, or alkylene (oligooxalkylene) group, optionally containing one or more fluorine substituents; and

Q is an ionic or ionisable moiety.

14. A composition according to claim 13 in which Q is selected from groups having the formula $-NR^{43}_p$, $-PR^{43}_p$ and SR^{43}_r, in which p is 2 or 3, r is 1 or 2, the groups R^{43} are the same or different and each is selected from the group consisting of hydrogen, C_{1-24} alkyl and aryl, or two of the groups

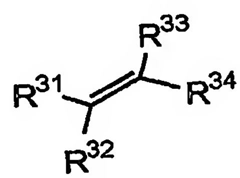
R^{43} together with the heteroatom to which they are attached from a 5 to 7 membered heterocyclic ring or three R^{43} groups together with the heteroatom to which they are attached form a 5 to 7 membered heteroaromatic ring, either of which rings may be fused to another 5 to 7 membered saturated or unsaturated ring, and any of the R^{43} groups may be substituted by amino or hydroxyl gruops or halogen atoms.

15. A composition according to claim 14 in which Q is $-NR^{43}_2$ where each R^{43} is the same and is C_{1-12}-alkyl, preferably ethyl.

16. A composition according to any of claims 13 to 15 in which B^1 is a C_{2-6}-alkanediyl, preferably $(CH_2)_{2-6}$.

17. A composition according to claim 8 or claim 13 in which the ethylenically unsaturated monomers include comonomer.

18. A composition according to claim 17 in which the comonomer has the general formula X



X

in which R^{31} is selected from hydrogen, halogen, C_{1-4} alkyl and groups $COOR^2$ in which R^2 is hydrogen and C_{1-4} alkyl;

R^{32} is selected from hydrogen, halogen and C_{1-4} alkyl;

R^{33} is selected from hydrogen, halogen, C_{1-4} alkyl and groups $COOR^2$ provided that R^{31} and R^{33} are not both $COOR^2$; and

R^{34} is a C_{1-10} alkyl, a C_{1-20} alkoxycarbonyl, a mono-or di-(C_{1-20} alkyl) amino carbonyl, a C_{6-20} aryl (including alkaryl) a C_{7-20} aralkyl, a C_{6-20} aryloxycarbonyl, a C_{1-20} -aralkyloxycarbonyl, a C_{6-20} arylamino carbonyl, a C_{7-20} aralkyl-amino, a hydroxyl or a C_{2-10} acyloxy group, any of which may have one or more substituents selected from halogen atoms, alkoxy, oligo-alkoxy, aryloxy, acyloxy, acylamino, amine (including mono and di-alkyl amino and trialkylammonium in which the alkyl groups may be substituted), carboxyl, sulphonyl, phosphoryl, phosphino, (including mono- and di- alkyl phosphine

and tri-alkylphosphonium), zwitterionic, hydroxyl groups, vinyloxycarbonyl and other vinylic or allylic substituents, and reactive silyl or silyloxy groups, such as trialkoxysilyl groups;

or R^{34} and R^{33} or R^{34} and R^{32} may together form -CONR^{35}CO in which R^{35} is a C_{1-20} alkyl group.

19. A composition according to any of claims 7 to 18 in which at least one of the blocks has a polydispersity of molecular weight less than 2.0, preferably in the range 1.1 to 1.4.

20. A composition according to any of claims 7 to 18 in which the degree of polymerisation of the ionic block is in the range 5 to 2000, preferably 10 to 250, and the degree of polymerisation of the zwitterionic block is in the range 2 to 1000, preferably 5 to 100, and in which the ratio of the degrees of polymerisation (ionic:zwitterionic) is in the range 1:5 to 10:1, preferably 1:1 to 5:1.

21. A composition according to any of claims 7 to 20 in which the or each block is formed by a living radical polymerisation process, preferably a group or atom transfer polymerisation process.

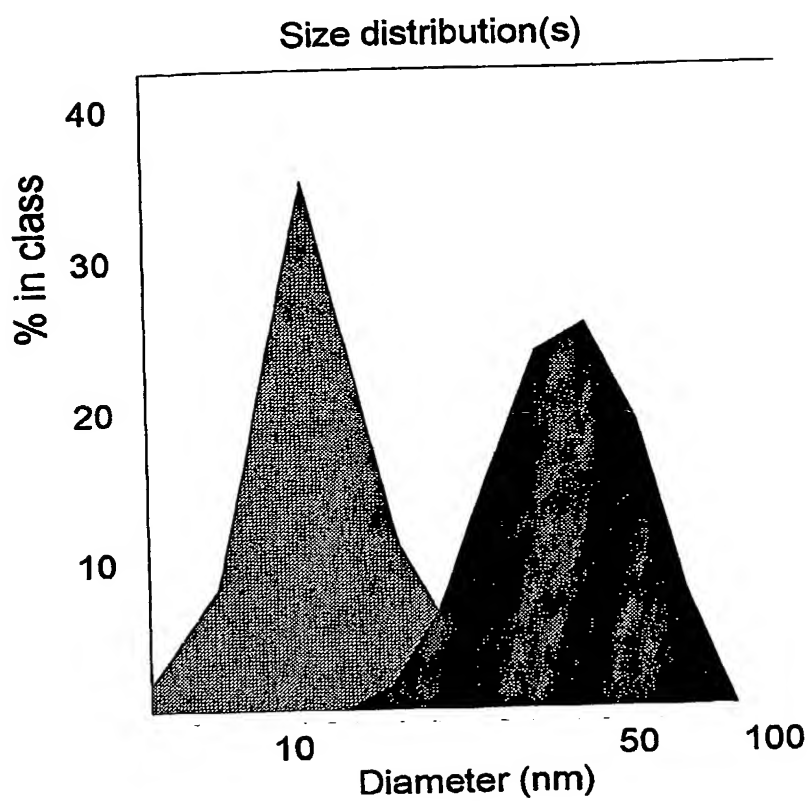
22. A composition according to any preceding claim in which the relative amounts of biologically active compound and polymer are in the range 1:5 to 10:1, preferably 1:2 to 5:2 based on equivalents of the polymer to active compound charged groups.

ABSTRACT

COMPOSITIONS OF POLYMERS

Liquid aqueous compositions are described comprising a suspension of a polymer having an overall ionic charge and pendant zwitterionic groups and a biologically active compound having a charge opposite that of the polymer. The polymer is preferably a linear block copolymer, preferably having a low polydispersity, such as a tertiary amino group containing monomer block-(zwitterionic monomer) copolymer. Suitable cationic monomers are dialkyl aminoalkyl(alk)acrylates and -acrylamides and suitable zwitterionic monomers are phosphorylcholine group containing acrylate monomers. The biologically active compound is generally polyionic and is for instance a nucleic acid, such as DNA, especially plasmid DNA.

Figure 1. Particle size (nm)



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